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Formulation and chracterization of meloxicam loaded niosome-based hydrogel formulations for topical applications

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Abstract

Analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) have gastrointestinal side effects and, particularly for local pain, topical dosage forms of these drugs are mainly preferred. The aim of this study was to develop meloxicam loaded niosomal hydrogel for enhanced transdermal and controlled drug delivery. Niosomal formulations were prepared by thin film hydration method using different types of non-ionic surfactant in the presence of cholesterol. Niosomal vesicles were characterized in terms of droplet size, zeta potential, surface morphology and entrapment efficiency. For enhanced residence time, niosomes were further loaded into the carbopol gel. The niosomal formulation containing Span 60, Tween 80 and cholesterol at a molar ratio of 6:1:0.6 had an optimally high percentage of drug entrapment with a mean vesicular diameter of 236.80 nm. Within 24 hours, a maximum of 46.83% drug release was achieved showing faster releasing profile than commercial meloxicam gel. Dermal and transdermal delivery of meloxicam using niosomal-gel formulations may offer promising alternative to traditional delivery systems of non-steroidal anti-inflammatory drugs with improved local and systemic but decreased adverse effects.

Keywords

Hydrogels, meloxicam, niosomes, topical drug delivery.

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INTRODUCTION

Meloxicam (MX) is a nonsteroidal anti-inflammatory drug (NSAID) that is structurally related to the 4-hydroxy-1,2-benzothiazine carboxamide enolic acid class. It was first approved by the United State Food and Drug Administration (US-FDA) in 2000 as a 7.5 mg tablet (Mobic- Boehringer Ingelheim). It was later approved and sold in capsule and suspension forms. These dosage formulations are used clinically to relieve acute and chronic pain and inflammation, as well as to reduce swelling, stiffness and discomfort due to arthritis. MX has also been studied as a possible treatment for Alzheimer's disease and as a potential adjuvant therapeutic chemotherapy agent for various tumors, including breast, colorectal, prostate and urinary bladder cancers, and has been found to have comparable but less toxicity than other NSAIDs to alleviate pain and inflammatory symptoms. In addition, MX is a drug with a low risk allergic reactions associated with NSAID intolerance (Ah *et al.,* 2010). For patients who are intolerant to other NSAID medications, MX is an efficient alternative drug. However, adverse reactions, such as gastrointestinal toxicity/bleeding, headaches, rash, increased risk of cardiovascular events are commonly reported when high-dose and long-term treatment of this medication is administered. Topical drug delivery is an alternative to oral administration, often with comparable effectiveness but theoretically with a more suitable tolerability profile. A variety of benefits over oral NSAIDs are offered by topical administration: These benefits are the ability to deliver the active ingredient more selectively to a given area with both local and systemic effects, the avoidance of first-pass effects, the elimination of gastrointestinal side effects and the improvement in patient compliance (Engelhardt *et al.,* 1995; Graeme, 2005; Noble and Balfour, 1996). Advancing technologies to promote the delivery of drugs to the skin site was a primary subject of study as the barrier function of the skin impairs the penetration and absorption of drugs by the skin barrier of the stratum corneum. The most advanced and less invasive methods for improved delivery of drugs through the skin barrier include a number of formulation strategies, such as micelles, liposomes, niosomes, and nanoparticles (Lengert *et al.,* 2020).

Niosomes provides comparable benefits to phospholipid vesicles (liposomes) and are capable of combining both water-soluble and lipid-soluble drugs as efficient drug delivery mechanisms for a wide variety of

applications and control releases. In addition, niosomes can be used as alternatives to liposomes that are both chemically and mechanically stable (Patel *et al.*, 2012). Niosomes may be formulated using basic methods and surfactants widely used in pharmaceutical technology. In case niosomes are introduced into vehicles such as hydrogels, the residence time for topical drugs will also be expanded (El-nabarawi *et al.*, 2015; Peppas *et al.*, 2000).

In this study, the goal was to investigate the formulations of niosomal hydrogel as potential carriers for the dermal delivery of MX. MX-loaded and gel-dispersed noisomes were subjected to structural tests and applied to *in vitro* release experiments.

MATERIALS AND METHODS

Materials

MX Span 60, Tween 20, Tween 80, Span 20, Carbopol 934P and cholesterol have been purchased from Sigma. Ethanol, ammonia, chloroform and regular saline have been purchased from Merck. Dialysis membrane filters have been obtained from Ashke Shishe, Tehran-Iran.

Preparation of niosomal vesicles

Using safe and non-toxic surfactants such as Tween 20, Tween 80 and Span 20, MX niosomes were prepared utilizing a thin-film hydration process followed by a sonification process (Figure 1). Cholesterol was used as a niosomal membrane rigidity enhancer (El-nabarawi *et al.*, 2015; Manconi *et al.*, 2002; Tavano *et al.*, 2013). For this study briefly, the ratios of non-ionic surfactants, cholesterol and MX that are listed in Table 1, were dissolved in 10 mL of chloroform: methanol: ammonia (3:1:1) mixture in a 100 mL round bottom flask. In a rotary flash evaporator at 45°C under reduced pressure (435 mbar), the flask was allowed to rotate for 15 minutes at 140 rpm to obtain a dry film.

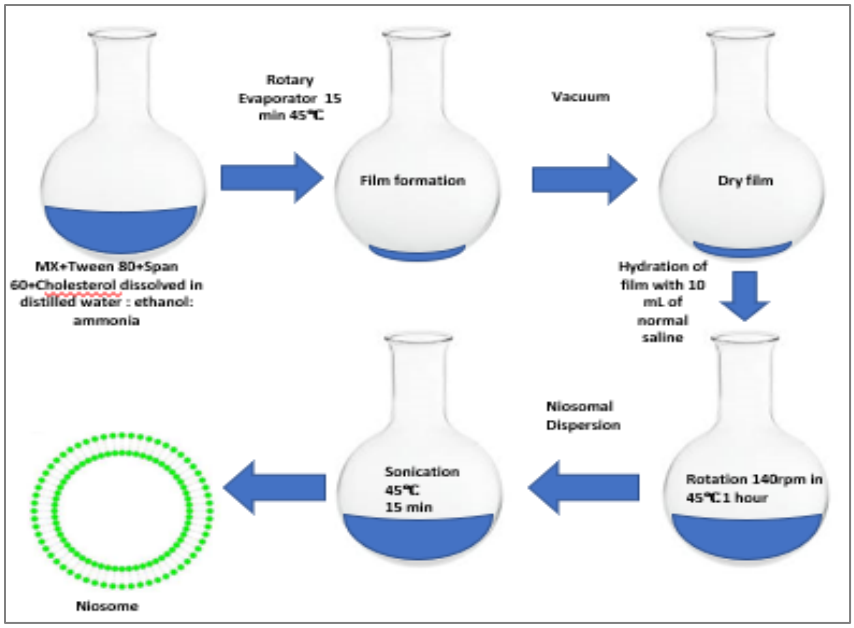

Figure 1: Procedures used for the formulation of MX niosomes using the thin-film hydration process.

This film was hydrated with 10 mL of saline solution and allowed under similar conditions to rotate further for 15 minutes. In a sonicator, bath the niosome dispersion was subsequently sonicated for 15 minutes at 45 °C (Ultrasons-HD 5 Selecta, Spain). The prepared niosomal dispersions were filtered through a 0.45 µm membrane filter to obtain transparent dispersions. The formulated formulations of vesicles (supernatant) were stored in airtight containers at 4°C prior to use.

Table 1: Meloxicam, non-ionic surfactant and cholesterol ratio used for the formulations.

	F1	F2	F3	F4	F5
Meloxicam	0.03	0.03	0.03	0.03	0.03
Tween 20	-	0.18	-	-	-
Tween 80	0.07	-	0.07	-	-
Span 20	-	-	-	-	0.02
Span 60	0.23	-	-	0.22	-
Cholesterol	0.05	0.06	0.02	0.1	0.02

Entrapment efficiency (EE%)

Standard stock solution of 1mg/mL MX was prepared for the calibration curve and validation of the assay method. Six working standard solutions with concentration of 1, 2, 5, 10, 20 and 30 µg/mL were prepared from stock solution and diluted with distilled water: Ethanol: Ammonia; 50 mL: 48 mL: 2 mL, respectively. The absorbances of resulting solutions were measured at λmax (362 nm) and plotted a calibration curve to get the linearity and regression equation.

Encapsulated MX was determined by ultra-centrifugation at 1500 rpm for 45 minutes (Hitachi/Cp100NX, Japan). Briefly, the supernatant was separated with a glass pipette and the precipitated vesicles were washed three times with distilled water to remove residual MX and surfactants in the environment. The incorporation efficiency was calculated from the collected niosomes by UV spectrophotometry (UV1800 Shimadzu Spectrophotometer, Japan) measured at λmax 362 nm, expressed as a percentage of the total amount of MX used initially (EE %).

EE% = amount of MX entrapped/total amount of MX *100

Vesicle characterization

The particle size and polydispersity index (PDI) of the niosomes were determined by a complex light-scattering method using the Malvern Zetasizer (Nano ZS, England). Vesicle formation and morphology were examined with optical microscopy by a camera attached to the optical microscope (Nikon HFX-DX, Japan) at 10×40 and 10× 100 magnifications.

Preparation of MX loaded niosomal-hydrogels

Carbopol-934 P (2.0% w/v) hydrogels containing MX-loaded niosomes equivalent to 1 % w/w of the drug were prepared by technique adopted by French *et al.* (French *et al.,* 1995). Through gentle mixing, a small portion of carbopol-934P was applied to the water. After the full inclusion of the polymer, hydrogel was naturally created by the addition of a few milliliters of triethanolamine. The formulated niosomes dispersed hydrogel was stored prior to use in airtight containers at 4°C.

Characterization of MX loaded niosomal-hydrogels

The rheological analysis of prepared niosomal-gel was evaluated using the Brookfield Digital Viscometer (DV-II, USA) at 37°C. Measurements at varying shearing speeds were applied for rheogram profile. In addition, pH measurements have been evaluated using an electronic pH meter (Jenway, U.K.). All experiments were done in triplicate.

***In vitro* dissolution studies MX loaded niosomal hydrogels**

In vitro drug release from the selected MX niosomal gel sample (F1) and market gel known as Ocam® (1% MX; Galeno) gel were investigated using semipermeable dialysis membrane filters (from Ashke Shishe, Iran). Membranes were hydrated by ethanol and ammonia over a night. 1% MX loaded niosomal hydrogel was inserted into dialysis membrane. Subsequently, filters were placed

within vessels containing 100 mL of release medium (distilled water: ethanol: ammonia; 50 mL: 48 mL: 2 mL respectively,) and stirred at 100 rpm at 37°C. In order to maintain the sink condition, samples of 1 mL of the receptor medium were replaced with 1 mL of the fresh receptor medium 24 hours (Qumbar *et al.,* 2017). Samples were analyzed utilizing spectrophotometry. In order to compare the dissolution profiles obtained in the release studies, the similarity factor (*f2*) and the difference factor *(f1*) were determined according to the SUPAC (Scale-up and post-approval changes) (FDA, 2017).

RESULTS AND DISCUSSION

Impact of formulation components on the encapsulation efficiency

The assay method was validated, and the analytical validation parameters (accuracy, precision, limit of detection, limit of quantification) were calculated. The linearity range of the method was 1–30 µg/mL with R2 of 0.9998. The limit of detection (LOD) was found to be 0.430 µg/mL and the limit of quantification (LOQ) was 1.302 µg/mL. The relative standard deviation for both intra-day and inter-day precision was less than 2%. Table 2 describes the results of encapsulation efficiency. F1 had the highest trapping efficiency of 56.00 % and F3 had the lowest trapping efficiency of 11.87%.

Table 2: Encapsulation efficiency results.

Formulation	Encapsulation efficiency (±SD)
F1	56.00 % (±0.85)
F2	22.12 % (±0.22)
F3	11.87 % (±0.17)
F4	37.98 % (±0.55)
F5	40.23% (±0.23)

The results shows that, relative to Tween-based formulations, Span 60 based niosomes had a substantially higher trapping performance ($p<0.05$). This may be due to Span surfactants' chemical composition. The increase in alkyl chain length could have resulted in greater efficiency of trapping. Span 60 has the longest alkyl saturated chain, which may be responsible for the highest efficiency of encapsulation (Hao *et al.,* 2002). Therefore F1, formulation was chosen for further characterization and assessment studies.

Impact of formulation components on the vesicle characterization

Optical microscopic images indicate that the resulting vesicles were almost spherical in shape and uniform in scale (Figure 2).

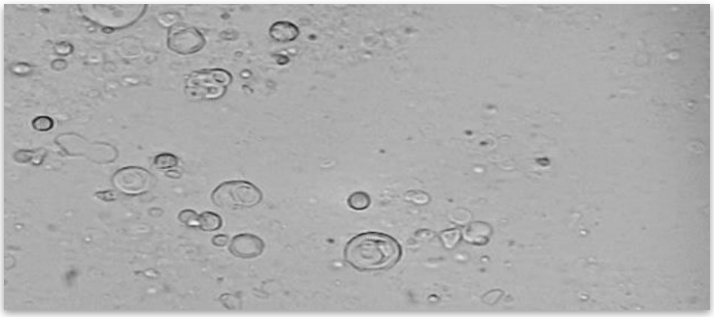

Figure 2: Optical microscopic image of prepared noisome vesicles.

The average size and PDI of the prepared niosomes are shown in Table 3.

Table 3: Average vesicle size and PDI of niosomal formulation.

Formulation	Mean particle size (nm) (±SD)	PDI
F1	236.70 (±2.21)	0.24(±0.06)
F2	350.00 (±0.75)	0.45(±0.03)
F3	384.00(±3.8)	0.52(±0.12)
F4	422.00(±3.2)	0.38(±0.04)
F5	301.00(±0.65)	0.30(±0.13)

The vesicle size distribution ranged between 0.24 and 0.65 with a narrow peak, indicating that this method produced relatively homogeneous vesicles. For all formulations, the particle size spectrum was observed to be between 236–422 nm. Based on the results, the scale of the niosomes showed a steady rise with an increase in HLB surfactant values. This is clearly observed with F1 formulation that consisted of Tween 80 (0.07; HLB: 15) and Span 60 (0.23; HLB: 4.7) (Figure 3). It is predicted that vesicles consisting of a mixture with a lower HLB surfactants values would have a smaller vesicle size than those with higher HLB values. This may be due to surface-free energy, since it decreases with increasing hydrophobicity (Gupta *et al.*, 2011; Nowroozi *et al.*, 2018; Sternberg and Florence, 1994)

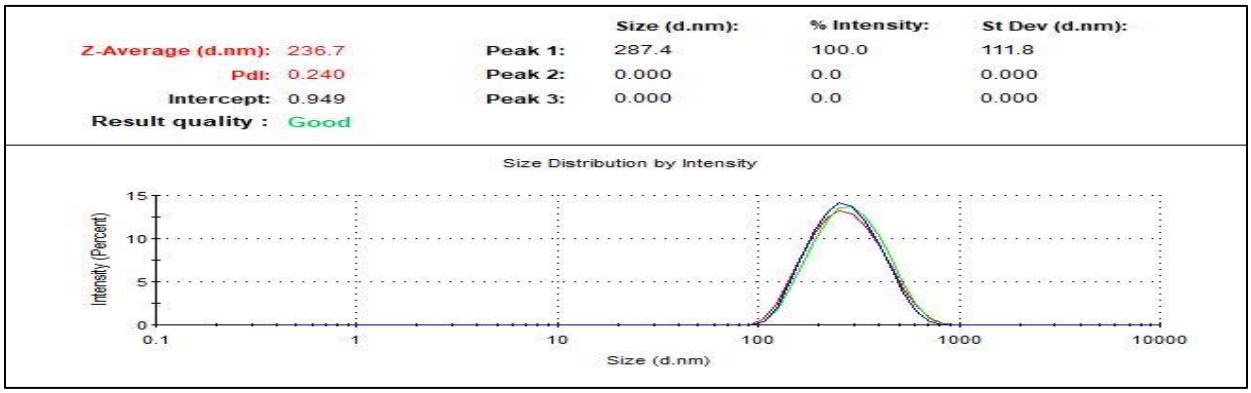

Figure 3: Vesicle size and PDI of F1 formulation niosomes measured with Malvern Zetasizer (Nano ZS, England).

Adequate findings were seen for the drug content, viscosity and pH of gel containing F1 niosomes as 97.4%, 244.66 cP, 7.1 respectively, (Figure 4).

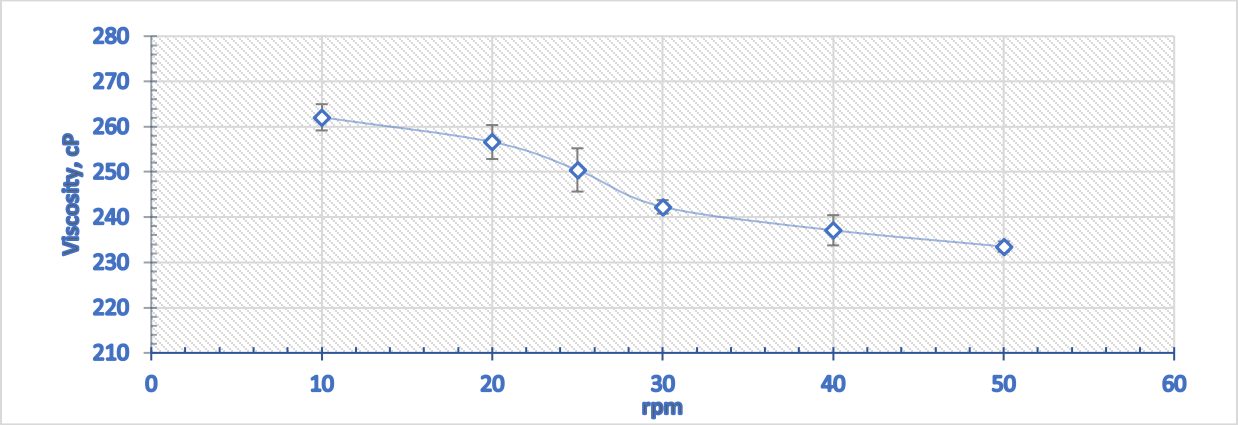

Figure 4: Viscosity profile of F1 niosome loaded hydrogel.

In vitro release of meloxicam from niosome based hydrogel

From pervious investigation, hydrogel loaded with formulation F1 niosomes was chosen for *in vitro* study because of its suitable size (236.7 nm), uniformity (PDI=0.240) and favorable entrapment efficiency (56.00%). The dialysis system was used to track, the amount of MX released from the vesicles within 24 hours. The release profiles of Ocam® (1 % MX gel) and MX niosome based hydrogel formulation are shown in Figure 5.

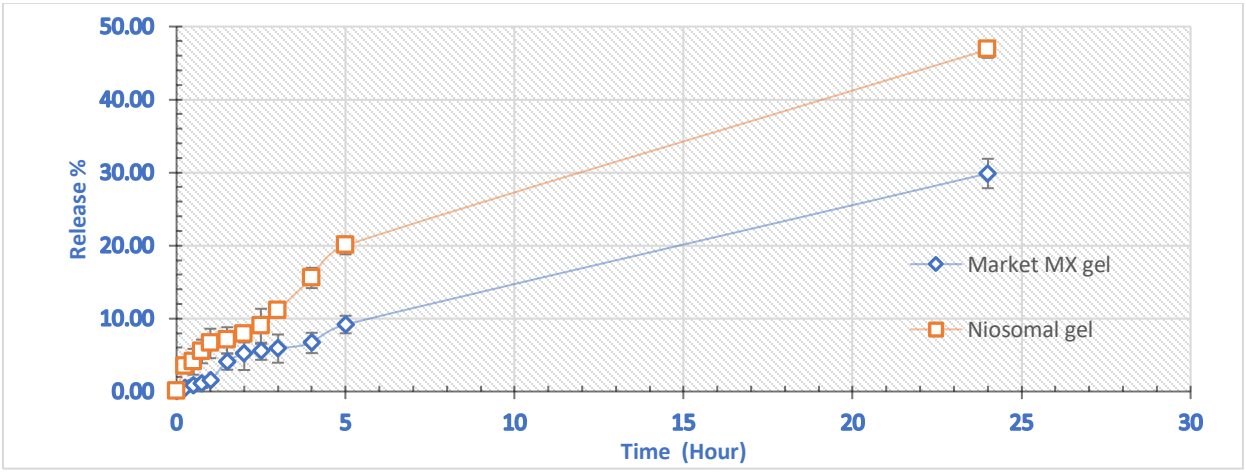

Figure 5: Comparative percentage release of MX from market product (Ocam®) and MX- niosome-based hydrogel.

Different methods have been published in the literature that can be used to compare dissolution profile data (Kassaye and Genete, 2013; Santos Júnior et al., 2014). The most commonly used tools, known as fit factors, have been used in this research. The fit

factors can be represented by two approaches: f1 (the factor of difference) and f2 (the similarity factor). In order for two dissolution profiles to regarded as identical and bioequivalent, f1 should be between 0 and 15, while f2 should be between 50 and 100 (Simionato et al., 2018). In this analysis, the dissolution profiles corresponding to the market product and the MX noisome-based hydrogel are found to be different according to this guideline (Table 4).

Table 4: Fit factors for Market product (Ocam®) and MX Niosomal-hydrogel.

Fit factor	Market product (Ocam®) -Niosomal gel comparation
f1 (the difference factor)	94.80
f2 (the similarity factor)	57.25

To predict the release trend of the drug from the MX niosome-based hydrogel, the *in vitro* release data was fitted to different release kinetics models. The findings indicated that the chosen formulation was best defined by Higuchi release kinetics (displaying the highest linearity and determination coefficient R2=0.976) suggesting that the concentration was independent of drug release.

CONCLUSION

High molecular weight and hydrophobicity of MX may restrict its tissue permeation for topical applications. Different types of non-ionic surfactants were used to prepare meloxicam-containing niosoms that could help MX to overcome this restriction. Hydrophobicity of surfactants has proven to play a role in the size of the niosomes. The devoloped niosome formulation showed a spherical shape, improved entrapment efficiency, and an acceptable polydispersity index and vesicle size. *In vitro* release studies have demonstrated a potential for improved topical delivery of the MX-charged niosomal-hydrogel formulation when compared to its commercial product.The present research has therefore successfully demonstrated the value of niosomal gel as an excellent delivery method for MX. More comprehensive animal and human trials should be undertaken to verify the potential of MX niosomal hydrogel for its anti-inflammatory activities.

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